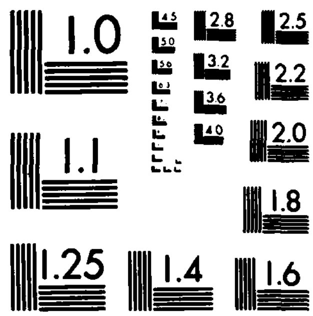
AD-A126 303 STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS(U) 1/1
ARMY MEDICAL RESEARCH UNIT NAIROBI (KENYA)
I E MURIITHI ET AL. SEP 81 DAMD17-80-G-9475

UNCLASSIFIED F/G 6/5 NL

END
DATE
FILMED
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD____________

(1)

STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS

Final Report

I.E. Muriithi
M.J. Reardon
B.T. Wellde
L.W. Roberts
J.D. Chulay
R.F. Beach
L.K. Lightner

September 1981

(For period 1 October 1980-30 September 1981)

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701

Grant No. DAMD17-80-G-9475

US Army Medical Research Unit--Kenya
Box 401, APO New York 09675

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

83 03 30 036

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A226 303 / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS	5. TYPE OF REPORT & PERIOD COVERED FINAL-- 1 Oct 80 - 30 Sep 81
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) I.E. Muriithi, M.J. Reardon, B.T. Wellde, L.W. Roberts, J.D. Chulay, R.F. Beach, L.K. Lightner	8. CONTRACT OR GRANT NUMBER(s) DAMD17-80-G-9475
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Unit - Kenya Box 401 APO New York 09675	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A802.00.067
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Ft. Detrick, Frederick MD 21701	12. REPORT DATE Sep 81
	13. NUMBER OF PAGES 26
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Africa, Kenya, Trypanosomiasis, T.b. rhodesiense, T. congolense, cattle, Glossina morsitans, Visceral Leishmaniasis, Kala-Azar, Leishmania donovani, Phlebotomus martini, sandfly colony.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

An outbreak of human sleeping sickness was investigated in the Lambwe Valley, Kenya. A total of 358 cattle and 500 people from the epidemic area were examined. Of 15 cattle showing central nervous system dysfunction, 13 were positive for Trypanosoma brucei sp., and all of these 13 had typical Trypanosoma rhodesiense - type brain lesions. Isolates collected from humans and cattle were cryopreserved for comparison with previous isolates from the area. Isolates from humans and cattle are being compared using neutralization techniques.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Immunologic studies were initiated with gamma-irradiated metacyclic forms of *T. brucei* and *T. rhodesiense*. Rats inoculated with 10 kr-irradiated parasites did not become parasitemic and were able to survive challenge one month after the final immunizing inoculation.

Mechanical transmission of polymorphic (blood form) *T. rhodesiense* trypanosomes by *Glossina morsitans* was demonstrated. Flies transmitted to rats up to 2 hrs 40 min after exposure on an infected donor. Active polymorphic trypanosomes were observed in fly proboscces for up to 5 hrs 20 min. post-exposure. The rate of mechanical transmission decreased with increasing time intervals post-exposure, but the parasitemia of the infected donor may be a more important factor in mechanical transmission.

Field studies were conducted to determine the epidemiologic parameters of visceral leishmaniasis in Kenya. Domestic dogs were investigated as a potential reservoir for human infection in the Turkana District, Leishmanial organisms were isolated from 1 of 38 dogs sampled from this area. Human isolates from Turkana are needed for enzyme comparisons with the dog strain and with other Kenyan strains. A total of 38 human cases of visceral leishmaniasis were identified from the Baringo District. Two of these human isolates were recovered in culture and are preserved in the Walter Reed - Nairobi leishmania storage bank. Active endemic foci of visceral leishmaniasis have been identified in Baringo District and will be studied during FY 82.

Field studies were conducted on sandfly species that are potential to determine the seasonal patterns of sandfly activity in Machakos District, Monthly collections of adult sandflies were plotted against rainfall. Distinct peaks of sandfly activity corresponded to the rainfall patterns. The importance of rainfall to sandflies using termite hill resting sites was demonstrated by comparing populations associated with artificially watered and unwatered termite hills during the dry season in Machakos District. Fourteen species of sandflies were collected from 2 areas where visceral leishmaniasis has been present historically. Five species of sandflies were reared in the laboratory in colonization attempts. F_1 adult flies have been obtained from 3 species, and F_2 larvae from a fourth species are now being reared. Successful laboratory colonization of these species has never been achieved previously. Colonies of sandflies are essential for evaluating vector potentials of these species. Collaborative taxonomic studies using enzyme and live sandfly biology are in progress and will be utilizing colony material.

A cryobank for preservation of leishmania isolates has been established. At present, 23 different isolates of *Leishmania* spp. are cryopreserved. Seven of these have been biochemically typed elsewhere and will be used for comparisons with other isolates in a cellulose acetate isoenzyme electrophoresis system. The other 16 isolates are of Kenyan origin, and one of these is being used in *in vitro* human macrophage studies and in biochemical evaluations. A suitable animal model system for Kenyan leishmania isolates is being sought.

A protocol was approved by the HSRRB for evaluation of high dose, short duration sodium stibogluconate (Pentostam(R)) in kala azar therapy.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

CONTINUATION OF SERODEME STUDIES - LAMBWE VALLEY, KENYA.

Introduction

Studies reported previously have documented a stable *T. rhodesiense* serodeme population in the Lambwe Valley, the site of the last remaining human trypanosomiasis focus in Kenya. During the past 10 yrs the number of human cases has averaged 15 per year. In 1980 this figure approached 100 cases. Dissections of 2,000 *Glossina pallidipes* showed that the cyclic infection rate with *T. brucei* group trypanosomes was 0.8%. It appeared that most of the infected flies were collected near the edges of the thornbush thickets closer to the villages and pastorage. This suggested a fly-domestic bovine-fly cycle of infection.

Progress

Teams collaborating with the Ministry of Health and Kenya Trypanosomiasis Research Institute surveyed both cattle and humans in the epidemic area. Of 358 cattle examined *T. brucei* sp. was isolated by mouse inoculation from 27 blood samples, 71 lymph node aspirates and 16 cerebrospinal fluid samples. Isolates of *T. brucei rhodesiense* were made from the blood of 64 humans and the cerebrospinal fluid of 6. Approximately 500 individuals were examined. All isolates have been cryopreserved.

During the survey 15 head of cattle were observed to have varying degrees of central nervous system dysfunction. These animals were purchased, examined and held for observation. Mice were inoculated with blood, lymph node aspirate and CSF. *T. brucei* type parasites were isolated from the CSF of 13 animals. All 15 were killed and post mortem examinations performed including microscopic evaluation of the brain. Brains from the 13 *T. brucei* positive cattle had lesions comparable to humans with sleeping sickness and to those described for experimental infections of *T. rhodesiense* in cattle. The 2 *T. brucei* negative animals did not have CNS lesions compatible with CNS trypanosomiasis. Naturally occuring CNS disease of cattle attributable to *T. brucei* has not been previously described. A manuscript is being prepared for publication.

Research Plan

The isolates from the present outbreak will be compared with those obtained from earlier surveys by previously described neutralization techniques. Isolates from humans will also be compared with those obtained from cattle.

IMMUNOLOGIC STUDIES WITH IRRADIATED METACYCLIC FORMS OF TRYPANOSOMA BRUCEI AND TRYPANOSOMA RHODESIENSE.

Introduction

Gray (1965) found that when T. brucei trypanosomes with variant antigens were ingested by tsetse flies the variants were replaced during cyclical development by trypanosomes with one particular antigen. This so-called "basic strain antigen" was present in mammalian hosts 3-7 days after being fed upon by infected tsetse flies (Gray, 1965). Similar findings were reported for T. gambiense (Gray, 1970). More recently, Stanley, et al. (1979) reported that one variant specific, presumably basic, antigen was found either as the predominant or minor antigen in all of 16 first peak parasitemia strains that they initiated with culture - or fly derived metacyclics. If basic strain antigen metacyclics can be inactivated such that they remain antigenic without producing disease, it is conceivable that hosts can be protected against challenge by infected tsetse flies. Gamma-irradiated T. rhodesiense blood forms induced resistance to homologous challenge in several host species (Duxbury and Sadun 1969; Duxbury et al. 1972; Wellde et al. 1973; Wellde et al. 1975). The objective of this study is to determine if irradiated metacyclic forms can induce protection in host

animals against challenge by *T. brucei* or *T. rhodesiense* infected tsetse flies.

Progress

Subsequent to the submission of the 1980-81 grant proposal the method of metacyclic production was restudied. It was decided to modify the protocol and to collaborate with Dr. M. Nyindo (International Centre of Insect Physiology and Ecology). An *in vitro* culture system is being used as the source of metacyclics (Nyindo et al. J. Parasitol. 1979).

A dose response experiment has been initiated to document the course of the disease in rats over a dose range of 10^1 - 10^6 parasites/rat. The radiosensitivity of cultured metacyclics is also being investigated. Aliquots containing 1×10^6 parasites/ml were irradiated over a dose range of 0 - 80,000 rad. Five rats were inoculated at each radiation dose level. All rats inoculated with 1×10^6 parasites subjected to 0 kr were parasitemic at 7 days post inoculation. One of five rats in the 5 kr group was parasitemic at 13 days. At 10 kr and above no rats developed patent parasitemias. While the trial is still underway it can be observed that the cultured metacyclics are both infective and apparently sensitive to relatively low level radiation.

At the conclusion of these preliminary experiments a definitive study will be undertaken to determine the immunizing effects, if any, of irradiated metacyclics. A preliminary syringe challenge experiment is presented in Table 1. A standard fly challenge system with an homologous isolate will be used against immunized animals in final evaluation of the metacyclic immunogen.

Table 1.

IMMUNIZATION WITH CULTURE FORMS

1st Challenge 1 wk after last Imm.	Prepatent period (Days)	Survival (Days)
4 "Immunized"	12, 13, 15, 50	23, 29, 29, 50
6 Control	8, 8, 8, 8, 8, 8	22, 23,23, 22, 21, 20
2nd Challenge 1 mo after last Imm.		
6 "Immunized"	None patent day 14	All alive day 14
3 Control	7, 7, 11	All alive day 14

MECHANICAL TRANSMISSION OF *TRYPANOSOMA BRUCEI RHODESIENSE* BY *GLOSSINA MORSITANS MORSITANS*

Introduction

Mechanical or direct transmission by tsetse flies of polymorphic *Trypanosoma brucei* subgroup trypanosomes has been demonstrated by several workers (Bruce et al. 1910; Duke 1921; Taylor 1930). However, the relative importance of mechanical transmission in the epidemiology of human sleeping sickness has not yet been determined. We studied mechanical transmission under laboratory conditions to determine the efficiency of *Glossina morsitans morsitans* as a mechanical vector of a human isolate of *Trypanosoma brucei rhodesiense*. Initial objectives were to (1) demonstrate direct transmission of a recently isolated strain from man and (2) determine the approximate duration of fly infectivity after exposure to polymorphic trypanosomes.

MATERIALS AND METHODS

The strain of *T. b. rhodesiense*, LVH 85 (Rosalina) was isolated from a patient by Kenya Ministry of Health personnel at the Homa Bay Hospital, Kenya, December 1980. The patient had been infected by fly bite in the Lambwe Valley, South Nyanza District, near Lake Victoria. A first passage isolate was used to infect 5 donor rats for mechanical transmission attempts. Parasitemias

of the infected rats were approximated by counting the number of trypanosomes per 100 leucocytes on thick blood smears and then relating these numbers to the total leucocyte counts per cubic millimeter of blood.

Two-day-old female flies that had never contacted a host animal were used in all experiments. For transmission attempts, individual flies were placed in stoppered, clear plastic tubes with screen mesh on one end. The mesh covered end of the tube was held against an infected donor rat for 30 seconds from the first probe or attempt to feed. The tube was then gently pulled away to interrupt feeding and each fly was later allowed to feed to repletion on a noninfected rat. The interval between exposure on an infected rat and the feeding to repletion on a normal rat was varied from 5 min to 24 hrs, with 20 individual flies tested at each interval. Wet blood films from the rats on which the exposed flies fed were examined daily for trypanosomes. Rats not parasitemic within 20 days after fly feeding were considered noninfected.

An additional 20 flies for each interval were dissected to determine if active polymorphic trypanosomes were present in the probosces. Each proboscis was placed in a drop of saline on a microscope slide and examined at 400X magnification. Approximate counts were made of the numbers of trypanosomes present.

RESULTS

Table 1 shows the results of proboscis dissections and frequency of mechanical transmission to rats at the various intervals

post-exposure. Active polymorphic trypanosomes were observed in the probosces of 35% (7/20) of the flies dissected 5 min post exposure but in only 2/20 (10%) of the flies dissected 5 hrs 20 min post exposure. Numbers of trypanosomes in the probosces varied from 1 to more than 400. We never observed trypanosomes in the proboscis without red blood cells from the infected donor also being present. Mechanical transmission was demonstrated up to 2 hrs. 40 min after exposure. At each post-exposure interval, up to 5 hrs 20 min, there were more probosces positive than there were transmissions to rats, but in both the dissection and transmission results, there was a general decline with increasing time intervals. The mean parasitemia of the donor rats ($\pm$ 1 SD) was $4.3(\pm 2.03) \times 10^5$ organisms per cubic mm.

DISCUSSION

From our results it appears that *G. morsitans* can mechanically transmit the polymorphic or blood form trypanosomes of *T. rhodesiense*. Several authors have suggested that mechanical transmission may occur under field conditions if the interval between exposure and second feeding is very short; however, we found that *G. morsitans* can mechanically transmit at least 2 hrs. 40 min after exposure. The parasitemia of the infected donor may be an important factor in mechanical transmission. Experiments are being conducted to determine the minimum parasitemia at which mechanical transmission can be demonstrated.

During the 1980 outbreak of human sleeping sickness in Lambwe Valley, dissection of 2,015 *Glossina pallidipes* showed a cyclic infection rate of only 0.8% with *T. brucei* subgroup trypanosomes. However approximately 50% of the cattle in the area had *T. brucei* subgroup infections. Since the people were in close proximity to the infected cattle and the fly population was high, the potential existed for direct transmission of *T. b. rhodesiense* from cattle to people. Tests are in progress to determine if G. morsitans can mechanically transmit *T. b. rhodesiense* from bovine-to-bovine.

If mechanical transmission of polymorphic trypanosome occurs under field conditions, the impact on vaccine development could be great,while a metacyclic - derived vaccine could be highly protective against cyclically infected flies, it might not protect against polymorphic trypanosomes directly transmitted from an infected host.

PROBOSCIS INFECTION AND DIRECT TRANSMISSION TO RATS FOLLOWING EXPOSURE OF INDIVIDUAL _GLOSSINA MORSITANS_ TO _TRYPANOSOMA BRUCEI RHODESIENSE_*

Interval Post-exposure	Proboscis Dissection No. positive/Total	(%)	Transmission No. rats/Total Infected	(%)
5 min	7/20	(35)	4/20	(20)
10 min	6/20	(30)	3/20	(15)
20 min	5/20	(25)	2/20	(10)
40 min	5/20	(25)	3/20	(15)
1 hr 20 min	4/20	(20)	1/20	(5)
2 hrs 40 min	4/20	(20)	2/20	(10)
5 hrs 20 min	2/20	(10)	0/20	(0)
10 hrs. 40 min	0/20	(0)	0/20	(0)

* $\bar{X}$ Parasitemia of Donor ($\pm$ 1SD) = 4.3 ($\pm$ 2.03) $\times 10^5$ mm^3

REFERENCES CITED

Bruce, D., Hamerton, A.E., Bateman, H.R. and Mackie, F.P. 1910. Mechanical Transmission of Sleeping Sickness by the Tsetse Fly. Proc. Roy. Soc. B 82: 498-505

Duke, H.L. 1921. On the Zoological Status of the Polymorphic Mammalian Trypanosomes of Africa and Their Relation to Man. Parasitol. 13: 352-397.

Taylor, A.W. 1930. Experiments on the Mechanical Transmission of a West African Strain of *Trypanosoma brucei* and *T. gambiense* by *Glossina* and Other Biting Flies. Trans. Roy. Soc. Trop. Med. Hyg. 24: 289-303.

PROBING BY <u>GLOSSINA</u> <u>MORSITANS</u> AND TRANSMISSION OF <u>TRYPANOSOMA</u> <u>CONGOLENSE</u> AND <u>TRYPANOSOMA</u> <u>RHODESIENSE</u>

Progress

Published in - Am. J. Trop. Med. Hyg., 30(5), 1981, pp 948-951

FIELD STUDIES OF VISCERAL LEISHMANIASIS

Introduction

Although surveys have previously been performed in areas of endemic leishmaniasis in Kenya, the vector(s), reservoir and natural history of the disease remains unclear. The projected studies will be closely tied to ongoing Ministry of Health disease surveillance surveys in Baringo District, the African Medical Research Foundation Hydatid Project - Turkana District, and the International Center of Insect Physiology and Ecology sandfly studies Kitui District.

Progress

Field work concerning some of the parasitological aspects of leishmaniasis in Kenya has been carried out in two areas of known Kala-azar endemnicity. The first of these is the Turkana District in extreme northwestern Kenya. Some of the earliest cases of human visceral leishmaniasis in Kenya were reported from this District, however due to the remoteness of the area and the nomadic habits of the people, little is known of the distribution and biology of the parasite in Turkana. Currently, the African Medical Research Foundation (AMREF) is conducting a research and control program for human hydatidosis in the Turkana District. Part of this program involves the killing and examination of dogs. Because

dogs have been incriminated as reservoirs of human leishmaniasis in various parts of the world, it was decided to take advantage of the AMREF program by examining the dogs for leishmania as well. An initial trip was made to Lokitaung in extreme northern Turkana in early March. Thirty-six dogs were examined by culture and impression smears of liver and spleen. Leishmanial organisms were isolated from a spleen culture of one of these dogs. This is the first isolation of leishmania from any animal in this part of Kenya. However, many more dogs need to be examined to determine the role of dogs as reservoirs of Kala-azar in Turkana. In addition, it will be necessary to obtain human isolates of leishmania from Turkana for enzyme comparisons with the dog strain and with human strains from other parts of Kenya. Further studies in Turkana are planned.

The second area currently under study is the Baringo District. Since early May 1981, the Division of Vector-Borne Disease (DVBD) of the Kenya Ministry of Health has been conducting an extensive screening survey for human Kala-azar in Baringo. USAMRU-K has been assisting both logistically and with personnel in this survey with an interest in developing the area for further vector and reservoir studies. To date, 5459 people have been screened by DVBD personnel. Of these, 1145 had a palpable splenomegaly. The majority of this latter group were then examined by splenic aspirate. A total of 38 cases of visceral leishmaniasis were identified from

various locations in Baringo. Two of these human isolates were recovered in culture and are preserved in the Walter Reed - Nairobi leishmania bank. In addition to screening by spleen palpation, extensive skin testing, using locally prepared leishmanin, was done. Positivity rates (indurations≥6 mm) varied from 5% to 22% depending on the location within the district. Results of both these surveys indicate that active endemic foci of visceral leishmaniasis are present in Baringo District and that the area should be examined for possible reservoirs of the parasites. Such studies are planned and will be initiated in October or November.

ENTOMOLOGICAL ASPECTS OF VISCERAL LEISHMANIASIS IN KENYA

Introduction

The objective of this study is to strengthen existing taxonomic knowledge of the Phlebotomine sandflies of Kenya, with emphasis on those species which vector visceral leishmaniasis. The project is to be carried out using laboratory-reared sandflies whenever possible, as it is felt that taxonomic information, derived from such "colonized" flies is missing from current keys to the sandfly fauna of the area. These missing data include:

1. Improved taxonomic drawings/measurements of each species: colonies will provide material which can be drawn 'fresh' rather than as fixed and mounted specimens, thereby providing distortion-free measurements of each species, something that cannot always be done with preserved material.

2. Zymogram 'fingerprints' for each species: ability to identify sandflies which are indistinguishable by conventional means (eg. males of the synphlebotomus group, all putative vectors of leishmaniasis, are indistinguishable) will be increased by including any detectable differences in enzyme electrophoretic mobility in the key.

3. Living taxonomic characters: comparison of live (colonized) specimens will provide 'living' characters, lost in killed/mounted material (eg. color, behavior, vectorial capacity).

RESULTS

FIELD WORK Collection of 'live caught' sandflies for colonization has been done at regular intervals over the past year, with emphasis on those species which rest in termite hills, where the vectors of leishmaniasis are known to reside. Collection data (species) analyzed over time also demonstrates the pronounced seasonality of termite hill-associated sandfly activity, and presumably disease transmission by these flies (Fig 1).

Question of the whereabouts of termite-hill associated sandflies during the dry periods was approached by a 10 week artificial watering experiment conducted during Dec., Jan. and Feb. of 1981 the results are summarized in Table 1. The ability to stimulate sandfly production during this period (which can be up to 1/2 of each year when the rains fail, as was the case this year) would improve the chances of routinely capturing enough live females to sustain and build a laboratory colony. The prolonged dry season and associated disappearance of sandflies also points out the problem of relying on field captured sandflies for entomological investigations.

While the number of P. martini, the most desirable species to have in colony, collected in watered hills during the experiment was low it is interesting to note that the same hills produced virtually no P. martini during the following rainy season (Mar-Jun). During this period more than 5000 specimens were collected from

these as well as other termite hills, suggesting that the natural martini population of the area was depleted. Therefore, watering at other locations may hold some promise as a means of stimulating termite-hill sandfly production during the dry season.

The first rains of 1981 (Mar-Jun) generated a large natural population of sandflies. Collections were made in two locations during this time, Eastern Province (Machakos District) and Rift Valley Province, both areas where visceral leishmaniasis has been present historically. These collections yielded a surprising variaty of species some of which were subsequently used in the colonization work described below. Species collected during the rainy season included: P. martini, P. celiae, S. garnhami, S. bedfordi, S. affinis, P. squamipleuris, S. kirki, S. rodhani, S. ingrami, S. schwetzi, S. multidens, P. guggisbergi, S. africana, and P. multidens, 14 of the 40 species reported in the literature as indigenous to Kenya.

LABORATORY WORK To date no Kenyan sandfly has been reared beyond the F_1 larval stages, that is beyond larvae hatched from eggs laid by wild caught (Parental generation) females. There are several points in the life cycle of the fly that act as barriers to colonization, where the mortality rate of the 1st generation becomes so high that the line dies out and must be started again from field collected material (always the blood fed female fly, larval stages of termite hill flies are unknown - an additional investigation

which can be done with colony reared material). The present status of the USAMRU-K colony is summarized in Table 2.

It is obvious that colonization is a numbers game where the starting egg batches, derived from wild females, must be large enough to counter the mortality rates incurred at each barrier. New techniques, eg. larval media, modified rearing chambers, special blood-feeding procedures, are felt to account for any success we have achieved to date.

COLLABORATIVE EFFORTS WITH USAMRDC SUPPORTED INVESTIGATORS

A collaborative program has been initiated with Dr. David Young, a sandfly taxonomist at the University of Florida, Gainesville. Dr. Young visited USAMRU-K this year and will be working on material from our colonies to produce an updated pictorial key to the sandflies of Kenya. USAMRU-Kenya will concentrate on enzyme and live sandfly biology to compliment this effort.

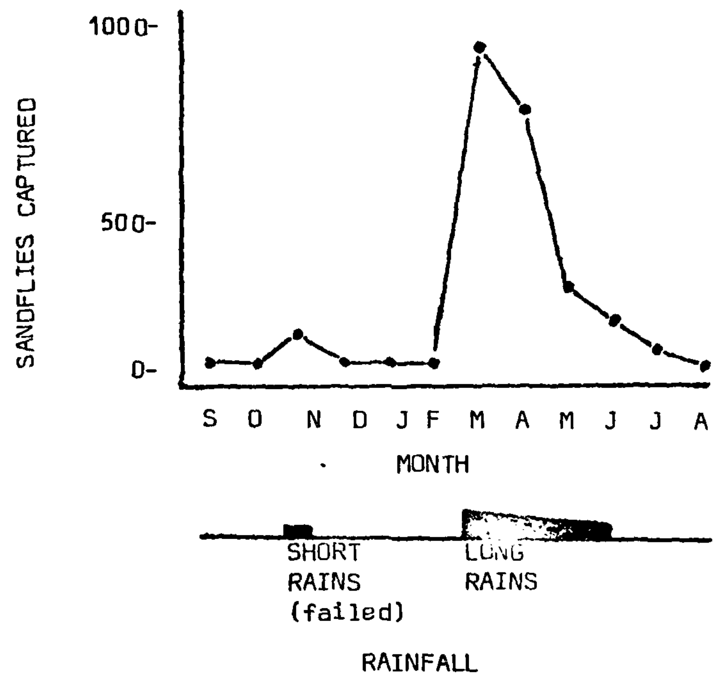


Figure 1. Monthly sandfly incidence and rainfall pattern, Machakos District. Includes flies caught in oil, light and flight traps as well as those captured live for colonization attempts. Annual total: 2251 sandflies

Table 1.

SANDFLIES ASSOCIATED WITH WATERED AND UNWATERED TERMITE HILLS *DURING THE DRY SEASON*, MACHAKOS DISTRICT

SPECIES	TOTAL NUMBER OF FLIES COLLECTED	
	EXPERIMENTAL HILLS (WATERED)*	CONTROL HILLS (UNWATERED)
Phlebotomus martini	20	0
P. rodhani	525	2
Sergentomyia bedfordi	446	20
S. schwetzi	25	2
S. squamipleuris	12	0
S. affinis	5	0
S. antennatus	7	0
TOTAL	1040	24

* APPROXIMATELY 40 LITERS OF WATER APPLIED TO TERMITE HILL DAILY

Table 2.

STATUS OF COLONIZATION ATTEMPTS USING FIELD COLLECTED KENYAN SANDFLIES

SPECIES	BARRIER EVENTS					STATUS
	PRE-OVIPOSITION DEATH OF WILD CAUGHT FEMALE	EGG STAGE MORTALITY[1]	LARVAL MORTALITY[2]	ADULT MORTALITY		
				PRE BLD[3] MEAL	POST[4] BLD MEAL	
	(observed mortality of flies surviving to event indicated)					
P. MARTINI	30%	10%	2%	--	--	F_1 adult flies now in the laboratory. Species has never been reared beyond F_1 larval stages. High mortality in the 1st instar.
P. GUGGISBERGI	40%	20%	100%			Colony died during adult emergence reason unknown.
S. BEDFORDI	50%	20%	10%	30%	75%	Large field collections have overcome very high F_1 mortality at each barrier. Species has never been reared beyond the F_1 larval stages. F_2 larvae now in the laboratory.
S. ANTENNATUS S. AFRICANUS	30%	15%	20%	--	--	F_1 adult flies now in the laboratory. Both species never reared in colony.

1 % of total eggs not hatching

2 % of larval stages not surviving to adult

3 % of adults dead prior to blood meal

4 % of adults dead prior to oviposition

LABORATORY STUDIES OF L. DONOVANI

Introduction

Several activities including culture attempts of material obtained from hospitalized leishmaniasis patients, antigen production and the operation of a reference cryobank will be performed under local agreement.

Progress

Laboratory work this year has been concerned primarily with establishing a functioning parasitology laboratory for support of clinical and field studies and the establishment of a cryobank for preservation of isolates of leishmania made in these studies. In addition studies were begun to determine suitable animal models for local leishmania strains.

At present the Nairobi Leishmania Bank has a total of 23 different isolates of *Leishmania* spp. cryopreserved. Seven of these are reference strains obtained from the WRAIR Leishmania cryobank and the WHO Leishmania Reference Center bank in Israel. These have been biochemically typed and will be used for comparisons in a cellulose acetate isoenzyme electrophoresis system which will be set up in Nairobi. The remaining banked isolates are of Kenyan origin. One of these has been inoculated into Walter Reed mice resulting in a patent infection and several

isolations from peripheral blood. One of the mouse isolates is currently being used in *in vitro* human macrophage studies and also in biochemical studies in conjunction with the Department of Biochemistry, University of Nairobi. However, initial results indicate that infections in the Walter Reed strain of mouse (currently used in USAMRU-K trypanosome studies) are not uniform enough to use as an animal model for leishmaniasis. Further studies using this mouse, BALB/c mice and hamsters with several isolates of Kenyan leishmania are continuing.

DISTRIBUTION LIST

Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
ATTN: SGRD-UWZ-C
Washington, DC 20012

Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick, Frederick, MD 21701

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDA
Cameron Station
Alexandria, VA 22314

DATE
FILMED